# INCIDENCE AND LEVELS OF *CAMPYLOBACTER* IN BROILERS AFTER EXPOSURE TO AN INOCULATED SEEDER BIRD

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## SUMMARY

***Campylobacter* is a very important human foodborne pathogen that is present in commercial broiler flocks. This organism lives in the intestinal tract of broilers without causing avian disease. However, commercial poultry products are frequently implicated as vehicles of human campylobacteriosis. Flocks are typically free of *Campylobacter* for the first 2 to 4 wk of broiler production. A flock may be completely free of *Campylobacter* 1 wk but entirely colonized by the next week. This study showed the incidence and level of *Campylobacter* transmission to pen mates of various ages after exposure to a single colonized seeder bird. Approximately 1 wk after being housed in a pen with an inoculated seeder bird, all of the other birds became *Campylobacter* positive. This work emphasizes the importance of preventing all sources of *Campylobacter* from a flock, because a single colonized bird could infect an entire flock during the growout period.**

**Key words: *Campylobacter*, broiler, seeder bird, age**

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## DESCRIPTION OF PROBLEM

*Campylobacter* enteritis is the leading cause of bacterial induced diarrheal disease in the United States and worldwide [1, 2]. The major vehicle transmitting *Campylobacter* to humans is poultry [3]. *Campylobacter* present during production and transport [4] is not usually eliminated in the processing plant [5]. *Campylobacter* colonization in broilers is associated with many factors, one of which is the age of the chicken [6]. Commercial broiler chicks typically become colonized with *Campylobacter* at between 2 and 4 weeks of production [7]. Presently, there is a lack of published information concerning the susceptibility of different age groups of broilers to *Campylobacter* colonization. Measures to control transmission through a flock may be more effectively applied if factors influencing *Campylobacter* colonization in chickens were better defined and understood. The goal of this study was to determine the effect of bird age on *Campylo-*

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*bacter* transmission after exposure to a colonized seeder bird.

## Materials and Methods

Day-of-hatch broiler chicks (350) were purchased from a commercial broiler hatchery and transported to our rearing facilities. Chicks were housed in isolation floor pens (70 birds/pen) measuring 7 ft. × 7 ft. × 7 ft. with filtered air and temperature control maintained by an electronic thermometer sensor. Fresh pine wood shavings were used as litter. Chicks were initially provided medicated broiler starter feed; from 3 wk until the end of the experiment, birds were fed a commercial broiler grower feed that contained a coccidiostat. Water was provided through nipple drinkers, and water and feed were consumed ad libitum.

To determine *Campylobacter* transmission rates within a flock of chickens of the same age, a seeder bird was introduced into a group of 70 *Campylobacter*-free pen mates that were 1, 2, 3, 4, or 6 wk old. Before introducing the seeder bird, five fresh droppings were collected to ensure that the flock was not already colonized by *Campylobacter*. Prior to being introduced into the naive flock, seeder birds were housed separately and inoculated by oral gavage with a suspension containing approximately 5 log cfu *Campylobacter* (cocktail of three strains isolated from naturally contaminated broiler carcasses) 2 d prior to being transferred to isolation units housing uninoculated birds. Three, five, and seven days after seeder bird exposure, 10 of the birds from each pen were killed, and ceca were collected and sampled to determine *Campylobacter* colonization.

Birds were killed by $CO_2$ asphyxiation before ceca were aseptically collected to determine incidence and level of *Campylobacter* colonization. The ceca of the dissected birds were mixed with phosphate-buffered saline (pH 7.2) at 1:3 (wt/vol) using a stomacher. Serial dilutions of cecal suspensions were surface plated onto Campy-Cefex agar [8] and incubated microaerobically (5% $O_2$, 10% $CO_2$, and 85% $N_2$) at 42 C for 24 to 48 h. Suspect colonies were selected and confirmed by phase-contrast microscopic observation of cells and by using a latex agglutination test [9]. The lower limit of detection was 30 cfu *Campylobacter*/g of cecal contents. This entire experiment was replicated twice, and the data were subjected to chi-square tests for independence. Analysis of variance and Duncan's multiple-range test [10] were also performed.

## Results and Discussion

Our study was designed to determine the rate of *Campylobacter* transmission through a flock of previously uncolonized broilers. To accomplish this goal, we placed a colonized, head-spotted seeder bird into flocks of same-aged broilers. Incidence (Table 1) and level (Table 2) of *Campylobacter* colonization were determined for the flocks at varying ages: 1, 2, 3, 4, and 6 wk. The experiments were conducted in isolation floor pens on litter to provide a well-controlled environment and to mimic commercial production.

TABLE 1. Incidence of *Campylobacter* transmission to broilers of selected ages following 3, 5, or 7 days of exposure to inoculated seeder birds

| | DAYS OF EXPOSURE TO SEEDER BIRD | | | | | |
|---|---|---|---|---|---|---|
| | 3 | | 5 | | 7 | |
| AGE (wk) | No. +/no. sampled | % + | No. +/no. sampled | % + | No. +/no. sampled | % + |
| 1 | 4/20 | 20 | 15/20 | 75 | 20/20 | 100 |
| 2 | 12/20 | 60 | 19/20 | 95 | 20/20 | 100 |
| 3 | 14/20 | 70 | 20/20 | 100 | 10/10 | 100 |
| 4 | 8/20 | 40 | 14/20 | 70 | 19/20 | 95 |
| 6 | 5/20 | 25 | 19/20 | 95 | 20/20 | 100 |
| Total | 43/100 | 43[a] | 87/100 | 87[b] | 89/90 | 99[c] |

[a–c]Averages in the same row with different superscripts are significantly different ($P = 0.05$) based on chi-square tests for independence.

TABLE 2. Levels of *Campylobacter* ($log_{10}$ cfu/g cecal contents)[A] transmitted to broilers after 3, 5, or 7 d of exposure to inoculated seeder birds

| | DAYS OF EXPOSURE TO SEEDER BIRD | | |
|---|---|---|---|
| AGE (wk) | 3 | 5 | 7 |
| 1 | 0.8[a] | 5.4[a] | 7.6[a] |
| 2 | 3.6[bc] | 6.6[a] | 8.4[a] |
| 3 | 7.1[c] | 8.2[b] | 8.2[a] |
| 4 | 2.2[ab] | 5.0[a] | 7.4[a] |
| 6 | 1.2[a] | 6.3[a] | 8.2[a] |
| $\bar{x}$ | 3.0[X] | 6.3[Y] | 8.0[Z] |

[A]Determined by plating of cecal contents onto Campy-Cefex agar plates.
[X–Z]Averages in a row with different superscripts are significantly different ($P = 0.05$) by Duncan's multiple-range analysis.
[a–c]Values in a column with different superscripts are significantly different ($P = 0.05$) by Duncan's multiple-range analysis.

In this study, all birds exposed to a seeder bird, except for one, eventually became colonized with *Campylobacter*. After 5 d of exposure to a seeder bird, 3-wk-old birds were colonized at significantly higher levels ($P = 0.05$) (Table 2) when compared to flocks at 1, 2, 4, or 6 wk of age. With the exception of the 2-wk-old group, 3-wk-old birds had higher levels after 3 d of exposure. On average the incidence of *Campylobacter* colonization increased significantly throughout the sampling period. After 7 d of exposure to the seeder bird, all of the birds were equally colonized in terms of level and incidence, regardless of when the pen mates were exposed to the seeder birds (Tables 1 and 2).

None of the groups became 100% positive following 3 d of exposure to the seeder bird. Only 3-wk-old birds were 100% positive following 5 d of exposure, but all the age groups were 95 to 100% positive by the seventh day of exposure to the seeder bird.

At Week 3, birds were switched from a starter ration to a grower ration containing a coccidiostat but no antibiotics. This change was the only notable difference in husbandry practices that occurred during the third week of growout in this experiment.

Birds that are intestinally colonized by *Campylobacter* will shed the organism in their feces. Broilers ingest each others' feces. As birds consume droppings containing *Campylobacter* they inoculate themselves. This phenomenon has been documented in commercial flocks. Smitherman et al. [11] reported that when *Campylobacter*-positive samples began to be detected, all samples from the chicken flock (size: ca 10,000 to 20,000 birds) became positive within 7 d. Data collected in the present study demonstrated that if a heavily contaminated bird was present, then virtually the entire flock became colonized within 7 d. This pattern of uniform transmission occurred regardless of the age of the birds in the flock at the time the seeder bird was introduced into the flock.

Understanding how *Campylobacter* gets into and spreads through flocks increases our options for devising effective interventions to broiler intestinal colonization by this organism. These results underscore the necessity of limiting the prevalence and level of *Campylobacter* colonization by having demonstrated how quickly one heavily colonized bird can lead to the colonization of the entire flock.

# Conclusions and Applications

1. Incidence and colonization levels increased with exposure time with almost 100% colonization and 100 million *Campylobacter*/g of ceca by Day 7 of seeder bird exposure.
2. These results demonstrate how rapidly broiler flocks can become colonized following only 7 d of exposure to a single heavily colonized seeder bird.
3. This study helps to explain the dramatic surge in broiler colonization by *Campylobacter* frequently observed in commercial broiler flocks following 3 wk of production.

# References and Notes

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